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Differences in sucrose metabolism relative to accumulation of bird-deterrent sucrose levels in fruits of wild and domestic *Vaccinium* species

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We examined variability in sucrose levels and metabolism in ripe fruits of wild and domestic *Vaccinium* species and in developing fruits of cultivated blueberry (*V. ashei* and *V. corymbosum*). The objective was to determine if sufficient variability for fruit sucrose accumulation was present in existing populations to warrant attempts to breed for high-sucrose fruit, which potentially would be less subject to bird predation. Threefold differences in fruit sucrose concentration were found among *Vaccinium* species, ranging from 19 to 24 mg (g fresh weight)$^{-1}$ in *V. stamineum* and *V. arboreum* to approximately 7 mg (g fresh weight)$^{-1}$ in cultivated blueberry (*V. ashei* and *V. corymbosum*) and *V. darrowi*. Hexose levels were similar among species, ranging from 90 to 110 mg (g fresh weight)$^{-1}$, and glucose and fructose were present in equal amounts. Soluble acid invertase (EC 3.2.1.26) activity was negatively correlated with fruit sucrose concentration. There was no apparent correlation between fruit sugar concentration and either sucrose synthase (EC 2.4.1.13) or sucrose phosphate synthase (EC 2.4.1.14) activities, both of which were low for all species studied. Developmental increases in fruit sugar levels of cultivated blueberry followed a pattern similar to that observed in fruit fresh weight accumulation. Hexose concentrations ranged from 6 to 30 mg (g fresh weight)$^{-1}$ during the first 60 days after anthesis. Between 60 days and fruit ripening (80 days), hexose levels rose from 30 to 80 mg (g fresh weight)$^{-1}$. Sucrose was not detected in fruits until ripening, when low levels were found. Insoluble acid invertase activity was relatively high early in fruit development, decreasing as soluble acid invertase activity increased. Between 60 days and fruit ripening, soluble acid invertase activity increased from 3 to 55 μmol (g fresh weight)$^{-1}$ h^{-1}. Both sucrose synthase and sucrose phosphate synthase activities were low throughout development. The extent of sucrose accumulation in fruits and the degree of variability for this trait among *Vaccinium* species support the feasibility of developing high sucrose fruits, which would be a potentially valuable addition to current strategies of minimizing crop losses to birds.

Key words – Bird predation, blueberry, fruit sugar, invertase, sucrose phosphate synthase, sucrose synthase, *Vaccinium* species.

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Introduction

Most fruits accumulate and store either sucrose or hexoses. Fruits such as peach (Moriguchi et al. 1991), mango (Hubbard et al. 1991), certain melons (Hubbard et al. 1989) and some citrus (Lowell et al. 1989) store relatively high levels of sucrose. However, small fruits, such as blueberries and strawberries, often store primarily glucose and fructose (Shaw 1988, Woodruff et al. 1960).

The physiological significance of this difference may

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be indirect, and may lie in the variation in capacity for sucrose digestion by seed dispersal agents. Many small fruits depend on frugivorous birds for seed dispersal, and a number of these bird species are unable to break down sucrose effectively. Several reports suggest that fruit traits may represent a response to selection pressures from seed dispersers, and that coevolution between specific fruits and specialized dispersers is probable (Martinez del Rio 1988, Reid 1991). Biochemical fruit traits are more likely to respond to selection pressures from dispersers than are other fruit traits (Reid 1991). Several researchers have suggested that the sugar composition of many fruits may have evolved in response to the taste preferences and/or digestive capability of the most effective seed dispersers (Martinez del Rio 1988 and others cited therein). The prevalence of hexose accumulation and storage in small fruits is consistent with this hypothesis.

The impact of sucrose vs hexose storage in fruits has been studied relative to the potential effect on subsequent seed dispersal. Studies with frugivorous bird species, such as the European starling (*Sturnus vulgaris*) and the American robin (*Turdus migratorius*) suggest that these birds exhibit feeding preferences for hexose sugars over sucrose (Brugger and Nelms 1991, Martinez del Rio and Stevens 1989, Martinez del Rio et al. 1988). In recent studies, starlings developed an aversion to sucrose solutions at concentrations as low as 0.17 to 0.22 *M* (Brugger et al. 1993, Martinez del Rio et al. 1988). Brugger and Nelms (1991) found that American robins reduced their total intake of sucrose-containing artificial fruits compared to glucose-containing fruits, suggesting that robins also developed an aversion to sucrose. The aversion in both species appears to be related to their lack of the digestive enzyme, sucrase, which is responsible for sucrose metabolism (Karasov and Levey 1990, Martinez del Rio and Stevens 1989). Thus, sucrose ingestion by starlings and robins produces an osmotic imbalance in the gut resulting in post-ingestional distress and a learned avoidance (Martinez del Rio et al. 1988).

Fruit producers throughout the United States face extensive bird damage to their crops. For example, yield losses to bird feeding and/or pecking in early-ripening blueberries in Florida approaches 75% in some years (Nelms et al. 1990). Bird damage to early-ripening cherries in New York can result in 60 to 100% yield loss (Tobin et al. 1991). Since early-ripening cultivars generally command the highest market prices, this translates into significant economic losses for producers. Methods used to deter bird feeding, such as noisemakers, traps and/or visual scare devices are relatively ineffective. Netting is the most effective method; however, it may be impractical and economically infeasible, especially for large areas (Avery et al. 1992). Thus, producers are left with few options to prevent or reduce bird damage.

Recent research in sugar metabolism of fleshy fruits has indicated genotypic differences in the type of sugar accumulated and stored. Theoretically, fruits such as blueberry can be bred for elevated sucrose levels if variability for this trait is present in existing populations. Studies with tomato (*Lycopersicon* spp.) strongly indicate this to be feasible. For example, some tomato lines (including the commercially grown *L. esculentum*) store hexoses, while related ones store sucrose (Yelle et al. 1988, 1991). Sucrose accumulation in tomato fruits appears to be controlled by a single recessive gene (Yelle et al. 1991), and is associated with low levels of acid invertase (EC 3.2.1.26) activity and protein (Klann et al. 1993, Yelle et al. 1988). Recent crosses have introduced the sucrose-accumulating trait into *L. esculentum* (Klann et al. 1993). The significance of such a change in hexose to sucrose storage may not be fully evident in fruits such as tomato, where crop losses to birds have not been a major problem. However, if high-sucrose blueberry genotypes can be identified, traditional breeding methods may be successful in developing commercial cultivars that accumulate bird-repellent levels of sucrose in their fruits. Furthermore, if the enzymatic basis for genotypic or developmental differences in sucrose accumulation in blueberry can be determined, then molecular approaches may be used to alter fruit sugar composition.

The primary objectives of the present study were to 1) assess the extent of existing genetic variability in sucrose and hexose levels in ripe fruits of several wild and cultivated *Vaccinium* spp., 2) determine the extent to which variability in fruit sugars may be attributed to developmental changes in fruits of two cultivated blueberry spp., and 3) determine the biochemical basis for genetic or developmental differences in sucrose accumulation.

Abbreviations – SPS, sucrose phosphate synthase.

Materials and methods

Plant material

Ripe fruits were harvested from wild *Vaccinium* species as well as from breeding lines and cultivars in the University of Florida germplasm collection. The species and breeding lines included several highbush cultivars (*V. corymbosum* cv. Flordablue, Sharpblue, O'Neal, Avonblue) and highbush selections; several rabbiteye cultivars (*V. ashei* cv. Bonita, Brightwell, Climax) and rabbiteye selections; selected lines of *V. darrowi*, *V. elliottii* and *V. australe*; and wild genotypes of *V. arboreum* and *V. stamineum*. Fruits from several clones within a genotype were harvested from the cultivars and selected lines. For the wild species, fruits were sampled from distinctly different plants at diverse locations, each probably representing an individual genotype. Harvested fruits were transported to the laboratory on ice and stored at −80°C until analysis. For examination of developmental changes, flowers from two commercial cultivars (*V. corymbosum* L. cv. Sharpblue and *V. ashei* Reade cv. Beckyblue) were tagged at anthesis, and flowers/fruits were harvested at 10-day intervals from anthesis through

fruit ripening. Fruit samples were handled and stored as above.

Sugar determination

Frozen blueberry fruits were ground in liquid N_2 and extracted in boiling 80% ethanol (1:10, w/v) to which 100 mg mannitol was added as an internal standard. Extracts were centrifuged, the supernatant decanted and the pellet re-extracted twice. The combined supernatant was partitioned against chloroform, and the aqueous fraction was dried under vacuum, resuspended in water, and passed through Dowex-1 and Dowex-50 ion resins (Sigma). The resulting fraction was dried under vacuum, resuspended in water, and passed through a 0.45-μm filter before injection into a Bio-Rad (Richmond, CA, USA) high-performance liquid chromatograph. Samples were analyzed for soluble sugars using a Bio-Rad HPX 87C cation-exchange column. Column temperature was 85°C and flow rate was 0.6 ml water min^{-1}.

Enzyme extraction and assays

Frozen blueberry fruit tissue (400–500 mg) was finely ground in liquid N_2, immediately transferred to a chilled mortar containing 5 ml of extraction buffer (50 m*M* 3-[N-morpholino]propanesulfonic acid [MOPS], pH 7.5, 5 m*M* $MgCl_2$, 2.5 m*M* 1,4-dithiothreitol [DTT], 1 m*M* ethylenediaminctetraacetic acid [EDTA], and 10% w/w polyvinylpolypyrrolidone [PVPP]), mixed thoroughly, and vacuum filtered. For soluble invertase assays, an aliquot of the filtrate was dialyzed (25 000 mol wt cutoff) for 24 h at 4°C against 5 m*M* K_2HPO_4 (pH 7.5). Soluble dialyzed extract was assayed for invertase activity as described below. The insoluble pellet was rinsed several times with 200 m*M* K_2HPO_4 (pH 7.5) and vacuum filtered prior to analysis. For sucrose synthase (EC 2.4.1.13) and sucrose phosphate synthase (SPS, EC 2.4.1.14) assays, a 1-ml aliquot of the filtrate was placed on 5-ml Sephadex G-50 (Sigma) columns equilibrated with the extraction buffer without EDTA. Columns were centrifuged for 1 min at 1 000 *g*.

Soluble and insoluble acid invertase activities were assayed in a 500-μl volume consisting of 2 m*M* acetic acid (pH 4.5), 100 m*M* sucrose and 200 μl extract (or 20–50 mg insoluble pellet). Reactions proceeded for 15 min at 30°C and were terminated by addition of 250 μl of 400 m*M* K_2HPO_4, pH 7.5, followed by boiling for 2 min. Neutral invertase was assayed using the same reaction medium adjusted to pH 7.5 with 5 m*M* K_2HPO_4. Enzyme controls contained all assay components, but were terminated at 0 min. Glucose production was quantified by the glucose oxidase method (Sigma).

SPS and sucrose synthase activities were determined according to the methods of Hubbard et al. (1989) with slight modifications. Activity of SPS was determined in a 70-μl reaction volume containing 50 m*M* MOPS (pH 7.5), 14 m*M* $MgCl_2$, 10 m*M* Fru6P, 50 m*M* Glc6P, 7 m*M* UDPGlc and 45 μl extract. Mixtures were incubated for 30 min at 30°C, then terminated with 70 μl 30% KOH, followed by boiling. Sucrose production was quantified by the anthrone method (Van Handel 1968). Sucrose synthase (degradative direction) was assayed in a 500-μl volume containing 100 m*M* 2-(N-morpholino)ethanesulfonic acid (MES), pH 5.5, 3 m*M* NaF, 5 m*M* UDP, 50 m*M* sucrose and 200 μl extract. Reactions were terminated with 500 μl 100 m*M* tris(hydroxymethyl)aminomethane (Tris), pH 8.7 + 0.2% EDTA, followed by boiling. Uridine diphosphate glucose (UDPG) production was quantified by measurement of UDPG dehydrogenase-specific synthesis of NADH (Lowell et al. 1989). Enzyme controls contained all assay components, but were terminated at 0 min.

Results

Genotypic variations in sugars and sucrose-metabolizing enzymes

Threefold differences in fruit sucrose concentration were observed among *Vaccinium* species (Tab. 1). Hexose levels were similar among species, and glucose and fructose were present in approximately equimolar amounts. The cultivated blueberry fruits (*V. corymbosum* and *V. ashei*) and fruits from *V. darrowi* all had relatively low sucrose concentrations, ranging from mean levels of about 6 to 8 mg (g fresh weight)$^{-1}$. Sucrose levels in *V. elliottii* and *V. australe* fruits averaged about 13 mg (g fresh weight)$^{-1}$. Both *V. arboreum* and *V. stamineum* fruits had significantly higher sucrose concentrations,

Tab. 1. Sugar concentrations (mg [g FW]$^{-1}$) in ripe fruits of various *Vaccinium* species. Data are the mean of the indicated number of genotypes within each species (n) ± SE.

Species	n	Sucrose			Hexose
		Mean	Minimum	Maximum	Mean
V. corymbosum	6	8.1 ± 0.4	6.80	9.90	107.1 ± 2.9
V. ashei	5	5.7 ± 2.1	2.30	4.30	93.7 ± 1.6
V. arboreum	5	19.0 ± 2.0	12.10	24.70	92.0 ± 3.4
V. stamineum	11	24.4 ± 2.7	16.50	45.30	96.7 ± 1.8
V. darrowi	5	7.7 ± 2.7	3.20	18.20	108.6 ± 6.7
V. elliottii	8	13.8 ± 1.3	9.80	20.40	109.1 ± 4.3
V. australe	8	12.4 ± 2.4	3.20	22.20	92.9 ± 4.6

Tab. 2. Sucrose-metabolizing enzyme activities in ripe fruits of various *Vaccinium* species. Data are means (± SE) of at least 3 genotypes per species and 2 replications per genotype, except for *V. arboreum* in which one genotype was tested.

Species	Soluble acid invertase (μmol [g FW]$^{-1}$ h^{-1})	Insoluble invertase (μmol [g FW]$^{-1}$ h^{-1})	Sucrose synthase (μmol [g FW]$^{-1}$ h^{-1})	SPS (μmol [g FW]$^{-1}$ h^{-1})
V. corymbosum	35.2 ± 4.3	4.8 ± 1.1	1.5 ± 0.1	6.7 ± 3.1
V. ashei	26.5 ± 3.5	6.1 ± 1.1	0.5 ± 0.1	1.7 ± 0.7
V. arboreum	6.3 ± 2.9	12.9 ± 1.9	0.0	0.3 ± 0.3
V. stamineum	0.9 ± 0.4	0.7 ± 0.2	0.2 ± 0.04	1.0 ± 0.6
V. darrowi	9.4 ± 4.0	2.3 ± 0.3	0.2 ± 0.2	0.9 ± 0.5

averaging 19 and 24 mg (g fresh weight)$^{-1}$, respectively. There were also distinct differences in the range of fruit sucrose levels among different genotypes within a species (Tab. 1). For example, there was a nearly threefold difference in fruit sucrose concentrations among the 11 *V. stamineum* genotypes analyzed, ranging from a low of 16 to a high of 45 mg sucrose (g fresh weight)$^{-1}$.

Blueberry species with high-sucrose fruits (*V. arboreum* and *V. stamineum*) vs low-sucrose fruits (*V. ashei*, *V. corymbosum* and *V. darrowi*) were selected for analysis of sucrose-metabolizing enzymes. No soluble neutral invertase activity was detected in extracts of fruits from any species (data not shown). Soluble acid invertase activity was highest in fruit extracts from the cultivated species, averaging about 26 and 35 μmol (g fresh weight)$^{-1}$ h^{-1} for *V. ashei* and *V. corymbosum*, respectively (Tab. 2). Activity was significantly lower in fruits of the other species, ranging from about 1 to 9 μmol (g fresh weight)$^{-1}$ h^{-1}. Insoluble invertase activity was highest in fruit extracts of *V. arboreum* and lowest in *V. stamineum*. Sucrose synthase activity was low or nondetectable in samples from all species tested. SPS activity was low for all species, despite the use of precautions suggested by Hubbard et al. (1989), and maximum activity of about 7 μmol (g fresh weight)$^{-1}$ h^{-1} was measured in fruit extracts from *V. corymbosum* genotypes.

Soluble acid invertase activity was negatively correlated with fruit sucrose concentration ($r^2 = 0.81$) (Fig. 1). There was no apparent correlation between fruit sugar concentration and any of the other sucrose-metabolizing enzymes.

Developmental variations in sugars and sucrose metabolizing enzymes

Blueberry fruits of both commercial species (*V. corymbosum* cv. Sharpblue and *V. ashei* cv. Beckyblue) exhibited similar patterns of growth, fruit sugar accumulation and sucrose metabolizing enzymes; therefore only the data for Beckyblue are presented. Fruit fresh weight accumulation followed a typical double sigmoidal growth curve (Fig. 2). Following an initial lag period after anthesis, fresh weight increased rapidly, leveling off between 30 and 40 days after anthesis. The final stage of rapid increase in weight occurred between 60 and 70 days after anthesis.

Developmental increases in total fruit sugar levels (Fig. 3) followed a pattern similar to that observed in fruit fresh weight accumulation. Fructose and glucose predominated and were present at approximately equal levels. Concentrations ranged between 3 and 15 mg (g fresh weight)$^{-1}$ during the first 60 days after anthesis. During the subsequent 10 days, however, glucose and fructose concentrations increased from about 15 to about 40 mg (g fresh weight)$^{-1}$, and did not increase further during ripening. Fruit sucrose concentrations were low

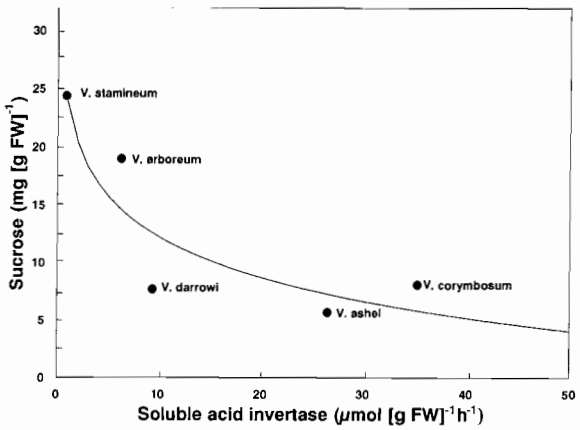

Fig. 1. Sucrose concentration in ripe fruits of selected *Vaccinium* species in relation to soluble acid invertase activity. $y = -5.11 \ln x + 24.03$, $r^2 = 0.81$.

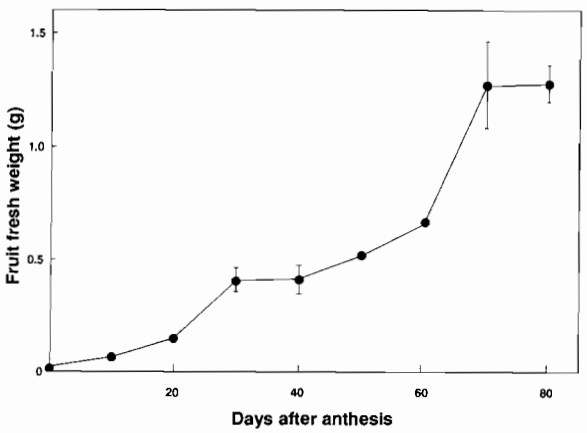

Fig. 2. Fresh weight of Beckyblue blueberry fruits from anthesis to ripening. Means ± SE. Error bars smaller than symbols not shown.

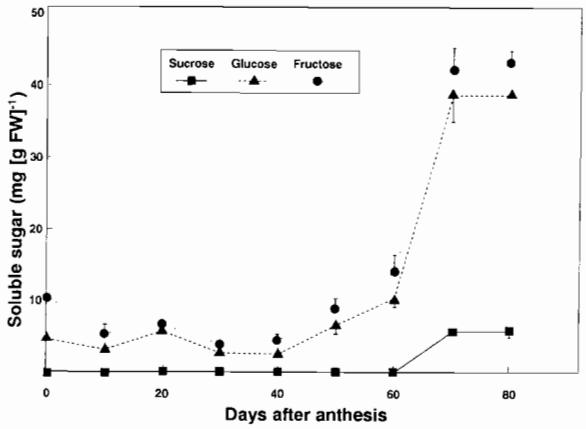

Fig. 3. Soluble sugar concentration in Beckyblue blueberry fruits from anthesis to ripening. Means ± SE. Error bars smaller than symbols not shown.

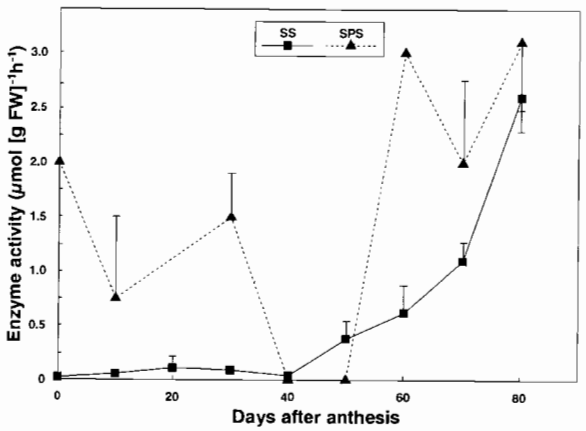

Fig. 5. Sucrose synthase and sucrose phosphate synthase activities in developing fruit of Beckyblue blueberry. Means ± SE. Error bars smaller than symbols not shown.

throughout development and increased only slightly (from 0 to about 6 mg [g fresh weight]$^{-1}$) between 60 and 80 days after anthesis.

Soluble neutral invertase activity was nondetectable throughout most of fruit development, increasing only slightly at ripening (Fig. 4). Insoluble acid invertase activity averaged about 16 µmol (g fresh weight)$^{-1}$ h^{-1} at anthesis, then gradually declined throughout most of fruit development. Activity of soluble acid invertase was negligible through 60 days after anthesis. Thereafter, activity in fruit extracts increased sharply, from about 3 to 55 µmol (g fresh weight)$^{-1}$ h^{-1}.

Both sucrose synthase and SPS activities were low in extracts from developing fruits (Fig. 5). There was, however, a small but significant increase in activities of both enzymes during fruit maturation, with the highest activities of about 3 µmol (g fresh weight)$^{-1}$ h^{-1} occurring at ripening. Although the increase was an order of magnitude lower than that observed in soluble acid invertase activity, both soluble acid invertase and sucrose synthase activities in the fruit extracts were highly correlated with fruit dry weight accumulation ($r^2 = 0.73$ and $r^2 = 0.84$, respectively; data not shown).

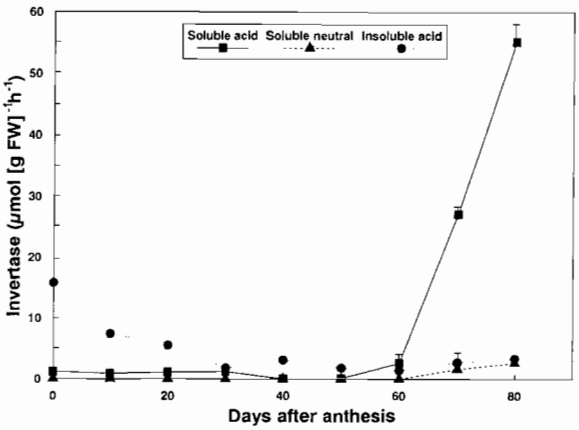

Fig. 4. Soluble acid, soluble neutral, and insoluble acid invertase activities in developing fruits of Beckyblue blueberry. Means ± SE. Error bars smaller than symbols not shown.

Discussion

Genetic variability

Fruit sucrose concentrations in *Vaccinium* species are highly variable, with some of the *V. stamineum* genotypes accumulating fruit sucrose to levels that have been reported to be bird deterrent (Martinez del Rio et al. 1989). Unlike other *Vaccinium* fruit, that of *V. stamineum* is not normally dispersed by birds, and usually abscises when ripe (P. Lyrene, personal communication). This observation is consistent with the suggestion by Cipollini and Stiles (1992) that fall-ripening Ericaceous fruits are less attractive to frugivorous birds than are summer-ripening fruit. Although they proposed that this may be due to differences in organic acids and phenol concentrations, it may also be due to increased sucrose concentration in fall-ripening species (i.e. *V. arboreum* and *V. stamineum*) compared to summer-ripening species (i.e. *V. corymbosum*, *V. ashei* and *V. darrowi*).

The existence of high-sucrose genotypes within the *V. stamineum* species, as well as the inherent variability for this trait in other *Vaccinium* species, suggests a reasonable probability for breeding blueberry fruits with high, bird-deterrent levels of sucrose. Genetic manipulation of hexose vs sucrose storage in fruits has recently been attempted in tomato for other reasons (Klann et al. 1993), and has resulted in the successful transfer of the sucrose-accumulating trait from a wild tomato relative (*L. chmielewskii*) into a background where almost 90% of the genome was contributed by the commercially produced tomato, *L. esculentum*.

Biochemical basis for variability

In many studies, sucrose accumulation in fruits has been correlated with the activities of one or more of the three sucrose-metabolizing enzymes. The present study indicates that *Vaccinium* fruit sucrose concentration is negatively correlated with activity of soluble acid invertase. Sucrose accumulation is rarely possible in the presence of active, soluble acid invertase, perhaps because both are often compartmentalized together in the vacuole, as suggested by Konno et al. (1993) and Leigh et al. (1979). A similar association between sucrose accumulation and lack of acid invertase has been found in studies of other fruits, ranging from peach (Hubbard et al. 1991) to muskmelon (McCollum et al. 1988) and tomato (Klann et al. 1993, Yelle et al. 1988). The capacity to accumulate sucrose in *Vaccinium* fruits appears to be mediated primarily by soluble acid invertase activity. Insoluble invertase activity did not correlate well with sucrose concentration. In addition, activity of insoluble invertase was lowest in *V. stamineum* and highest in *V. arboreum*, both of which accumulated high levels of sucrose.

In many studies, however, reduced invertase activity alone appears to be essential, but not always sufficient, for extensive sucrose accumulation. Hubbard et al. (1989) found that muskmelon genotypes that accumulated different amounts of sucrose had similar invertase and sucrose synthase activity, but differed in SPS activity. Although developing peach fruits contained no detectable acid invertase activity, sucrose accumulation during development began when sucrose synthase and SPS activities increased (Hubbard et al. 1991). In addition, the increases in sucrose levels in ripening muskmelon were correlated not only with decreased invertase activity, but also with increased sucrose synthase activity (McCollum et al. 1988). In tomato, Miron and Schaffer (1991) found that low invertase genotypes did not necessarily accumulate sucrose and that this trait seemed to require the additional presence of active SPS as fruit development progressed. Klann et al. (1993), however, indicated that SPS activity in fruits was not limiting sucrose synthesis in any of their high- or low-sucrose tomato genotypes. Activities of both sucrose synthase and SPS were low for all genotypes and species analyzed in the present work, and did not correlate with fruit sugar composition. Thus, the suggestion that high SPS may be characteristic of sucrose-accumulating tissues (Miron and Schaffer 1991) does not appear to hold true for *Vaccinium* fruits.

Developmental variability

The developmental increases in fresh weight paralleled those of soluble hexose accumulation in the fruits of two commercially cultivated species. The positive correlation between acid invertase activity, hexose accumulation and fruit growth suggests that acid invertase activity may play a major role in determining sink strength of *V. corymbosum* and *V. ashei* fruits. A similar relationship has been proposed in developing sweet pepper fruits (Nielsen et al. 1991), developing maize kernels (Hanft and Jones 1986) and germinating pinyon seedlings (Murphy et al. 1992). In other species, however, sink strength and sugar accumulation have been attributed to activity of sucrose synthase (Sun et al. 1992, Wang et al. 1993) or SPS (Hubbard et al. 1989, Miron and Schaffer 1991). All three enzymes may be active in young sink tissues (Lowell et al. 1989), and it is possible that invertase may contribute osmotic constituents and apoplastic gradients, while sucrose synthase provides entry into respiration and cell wall biosynthesis, and SPS is used to resynthesize sucrose for storage. Predominance of any given enzyme may reflect the balance between expansion, synthesis and storage, as well as the overall diversity in mechanisms by which sink organs compete for assimilates.

The relatively high activity of acid invertase associated with the insoluble fraction in young *Vaccinium* fruits may be important to both the capacity for, and the fate of, sucrose imported during initial development. Sucrose hydrolysis could maintain a gradient favorable to unloading, as suggested by Miron and Schaffer (1991). Later increases in soluble acid invertase in mature *Vaccinium* fruits are similar to increases observed in maturing papaya (Hubbard et al. 1991) and *L. esculentum* (Sun et al. 1992). This marked increase in soluble acid invertase activity may also be important in maintaining a sucrose gradient from source to sink. This would be consistent with either a symplastic unloading pathway or an apoplastic one in which sucrose is taken up without hydrolysis, then rapidly cleaved to hexoses, thus maintaining a sucrose concentration gradient between apoplast and symplast. Sucrose synthase activity (degradative direction) appears to play only a minor role in sugar accumulation in *Vaccinium* fruits. Although activity increases concomitantly with increasing fruit hexose levels, the maximum activity is an order of magnitude less than that of acid invertase. The slight increase in SPS activity observed in ripe fruit tissue is sufficient to account for the observed increase in fruit sucrose; however, the role of SPS in sucrose accumulation in *Vaccinium* fruits is unclear, since genetic variability in sucrose accumulation did not correlate with SPS activity.

Biological implications

The extent of sucrose accumulation in fruits and the degree of variability for this trait among *Vaccinium* species supports the feasibility of developing high-sucrose fruits. Nevertheless, questions remain as to the usefulness of this approach in reducing bird damage to small fruits. The impact of changes in fruit sugar metabolism and hence sucrose content will vary with the birds' capacity for sucrose metabolism. Species such as starlings and robins that lack sucrase will experience post-ingestional distress from eating the sucrose-rich fruits and respond by subsequent avoidance. On the other hand, species such as

the cedar waxwing (*Bombycilla cedrorum*) are tolerant of sucrose, yet are inefficient in its digestion. They are likely to respond by eating more of the sucrose-rich fruits in order to compensate for their digestive inefficiency (Avery et al. 1994). Although probable results must not be oversimplified, the production of sucrose-rich fruits can be viewed as a potentially valuable means of enhancing current strategies of minimizing crop losses. Overall, the present research describes a clear example of the potential for genetic alteration of fruit physiology for enhanced resistance to biological pests.

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